Classical Protein Chemistry in a World of Slicing and Splicing

The explosive development of DNA recombinant technology has made it possible to carry out large-scale production of a number of medically and industrially important proteins and peptides. At the same time, new methods in protein chemistry have appeared which should increasingly permit the efficient isolation, structure determination, and synthesis of large and small polypeptides.

Before much of anything can be done with a protein, it must be isolated in pure form and its covalent structure must be determined. The standard methods of protein isolation have been enormously facilitated over the past few years by the development of the technique of affinity chromatography using columns to which have been bound either substrate analogs or specific antibodies. In many cases, affinity chromatography can lead directly from the crude source of a protein to homogeneous material in a single step. At this point, we may capitalize on still another exciting development in modern protein chemistry - microsequencing as carried out strikingly by Hunkapiller and Hood at Cal Tech. Their improvements in the original method of Edman have led to the commercial availability of equipment that even novices can use with considerable success and, most important, on very small samples of material, frequently less that 50 picomoles. Having determined the amino-acid sequence of a protein and the nature of any non-aminoacid components attached to side chains, the protein can then be turned over to our crystallographic experts -- individuals such as Joe Kraut and his colleagues around the world who employ the new developments in heavy atom tagging and, with the availability of sophisticated equipment that efficiently feeds diffraction data into intelligent computers, can produce three-dimensional representations with outstanding speed.

My own particular interest over quite a few years has been the relation between the primary structures of protein molucules and their three-dimensional structure. Most of us are convinced by this time, that the three-dimensional structure is completely determined by the primary structure and that the folding process goes spontaneously.

Extensions of the folding problem have become particularly important in the last ten years or so because of interest in such possibilities as the preparation of totally synthetic

vaccines. Can one look at the structure of a protein, preferably in three dimensions, and select areas on the surface which might be expected to have a strong tendency to fold properly even when chopped out of the total structure? I will describe to you a few experiments relating to this biological problem in a few moments. My own interest began with some work that was carried out with Ruth Arnon and Michael Sela at the Weizmann Institute on the so-called lysozyme loop -- a small disulphide-stabilized extension on the enzyme. When this loop was prepared synthetically and attached to a non-immunogenic carrier, injection into animals produced antibodies which did indeed recognize the parent enzyme molecule. One might say that this material constituted a synthetic vaccine against the lysozyme molecule. Through the activity now going on in a variety of laboratories -- I might mention the Scripps Clinic in La Jolla, some work from which will be described to us by Dr. Getsoff; the Weizmann Institute in Rehovot, Israel; and our own laboratories at the NIH -- we became interested in the ability of various peptide fragments of proteins to fold up into the conformations that they assume when part of the native protein. Affinity chromotographic methods were employed to isolate antibodies against various parts of molecules, which could then be used to study the "foldability" fragments.

Let me conclude by mentioning a few bits of relatively recent protein chemistry that suggest that a good deal of methodology for the specific modification of proteins and partial or total synthesis is already available. Homandberg and Laskowski demonstrated, in the late 70's, that protein fragments could, following modification of the solvent to include high levels of a nonaqueous substance such as glycerol, be proteolytically reunited to produce the original parent chain. These stitching procedures are of considerable potential value since they provide a way of joining portions of protein chains that fall within the useful limits of the Merrifield solid-phase technique.

Professional Interests:

Protein Chemistry: synthesis, structure-function, folding, isolation, and chemistry of the interferon molecule.

Awards and Personal Interests:

Interesting punctuation points in my own career have been a Nobel Prize in Chemistry in 1972, a long-standing connection with the Weizmann Institute of Science in Israel, and an on-going fascination with (and I might say frustration with) the broad problem of human rights.